IN THE NAME OF GOD

CANCER CELL REPROGRAMING

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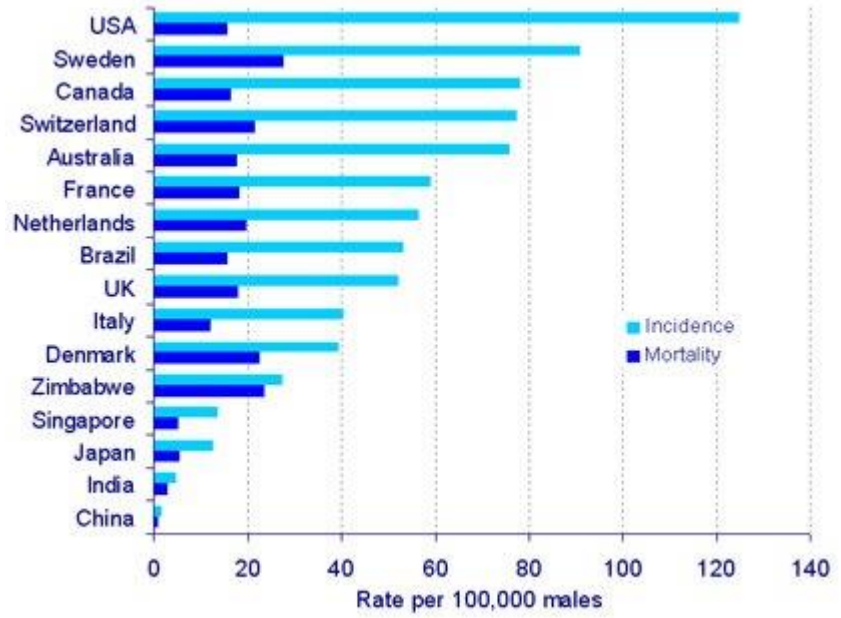
CONTENT:

- CANCER
- REPROGRAMMING
- OSKM
- microRNA
- PROBLEM AND ADVANTAGE
- REFRANCE

Cancer:

- **Disease of cells**
- **disruption of the orderly process of cell growth**
- **main causes of death after cardiovascular disease**
- **Cancer is the third main cause of death in Iran (1)**



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Cancer has two aspects:

- ✓ Environment (about 90 %)
- ✓ Genetics (about 10%)

- Environmental causes such as smoking, diet and obesity, radiation, infection, stress, lack of physical activity and environmental pollution

- Genetic factors can be classified into two groups:

1. Oncogenes → lead to cell growth
2. Tumor suppressor → an important role in controlling cell growth and division.

- **The most important oncogenes such as:**

ERK ,MYC,WNT,RAS and TRK*, FOS*, and *PML-RAR*

- **The most important Tumor suppressor such as:**

p15,p16,p18,p19,p21,p27 and p53

Genomic changes in cancer

↓

causing mutation

↓

Production oncogenic genes with dominant performance

↓

The loss of the tumor suppressor gene function

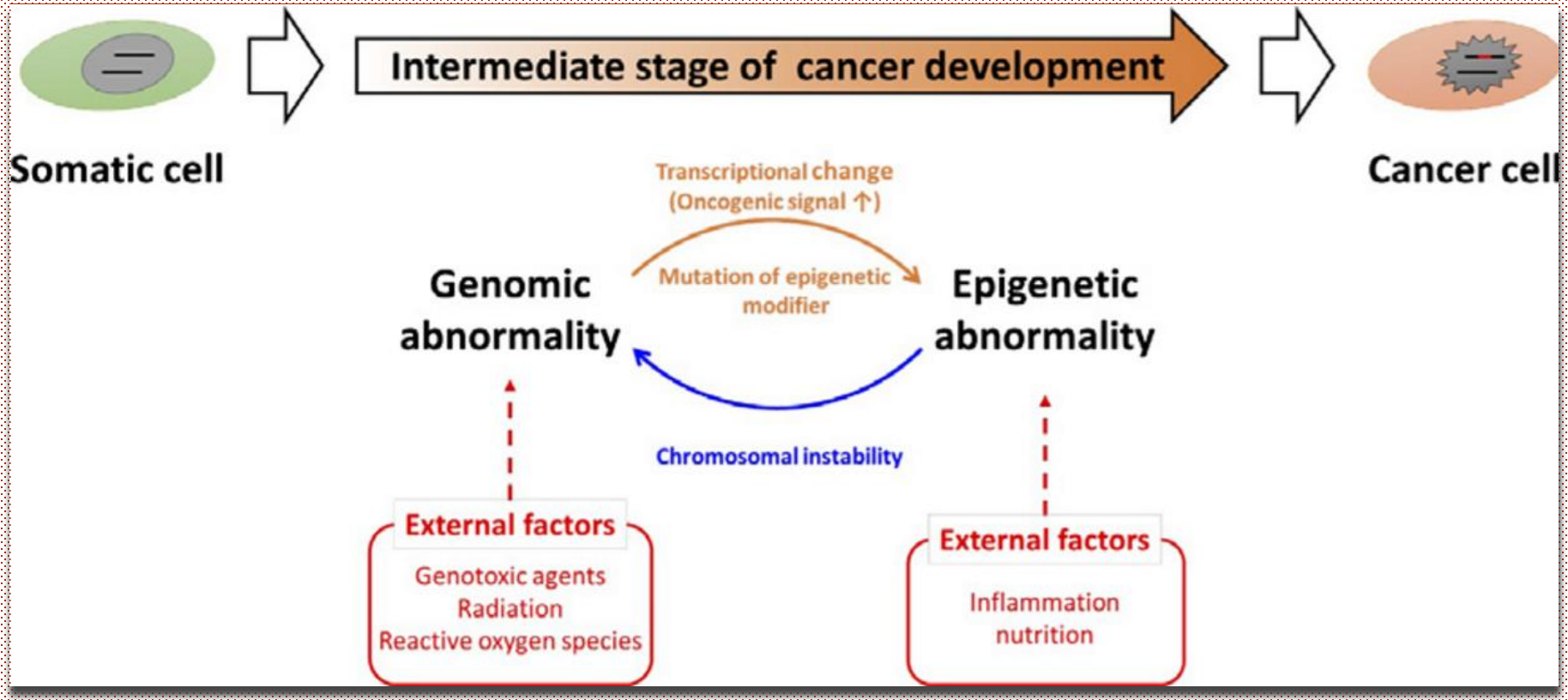
↓

abnormal and excessive cell growth

Other factors has a role in cancer development: (2)

✓ Epigenetic changes including hyper methylation and methylation in the DNA and histones, the acetylation/deacetylation of histones, and the packing of chromatin into euchromatic and heterochromatic regions

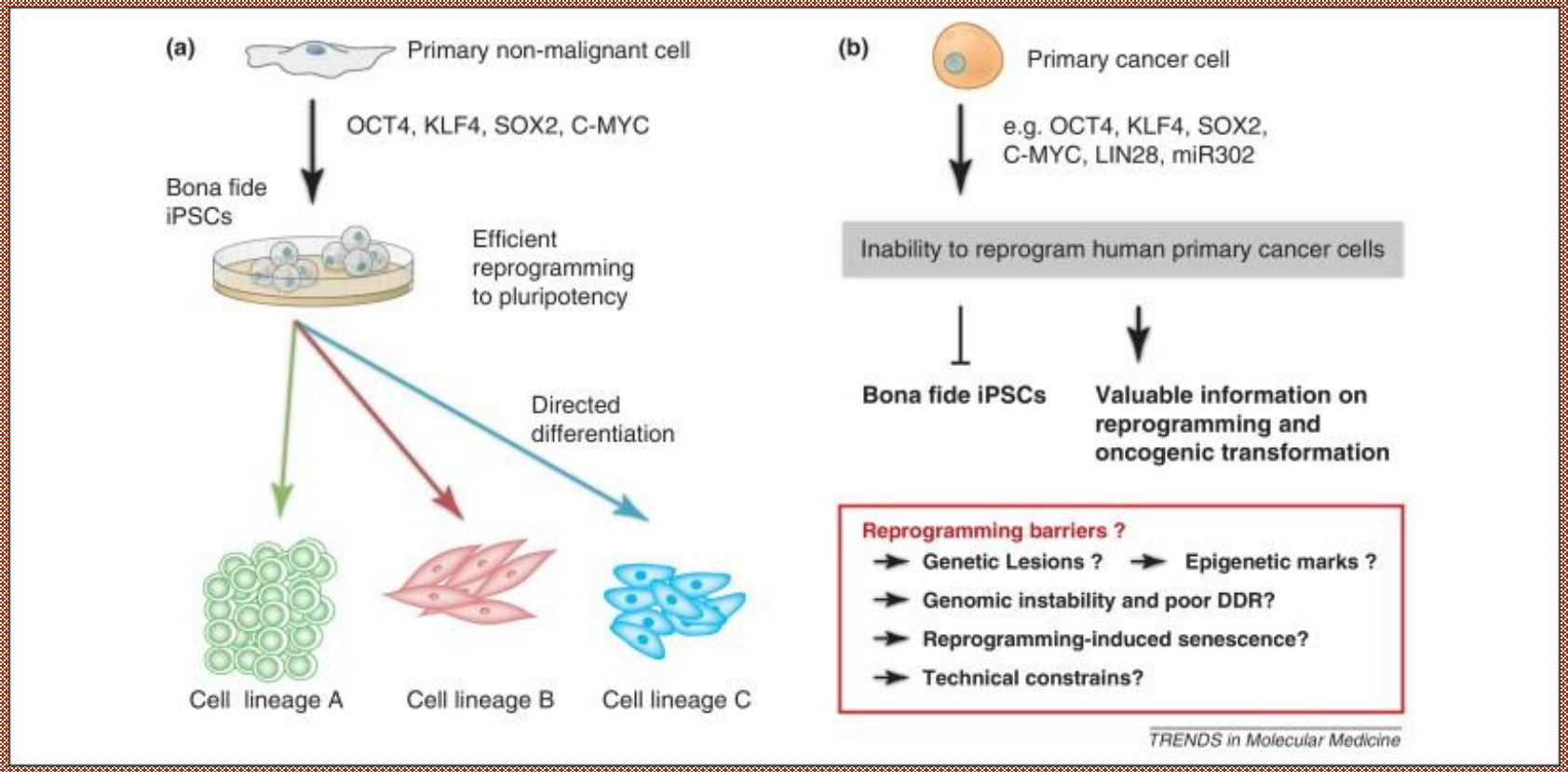
- reduced DNA methylation → chromosomal instability
- Hayper methylation DNA → silencing of tumor suppressor genes, such as *VHL*,*BRCA1* and *LKB1*.
- Irregularities in the cell cycle → A very important factor in the pathology of cancer.



Crosstalk between genomic and epigenetic abnormalities during cancer development.

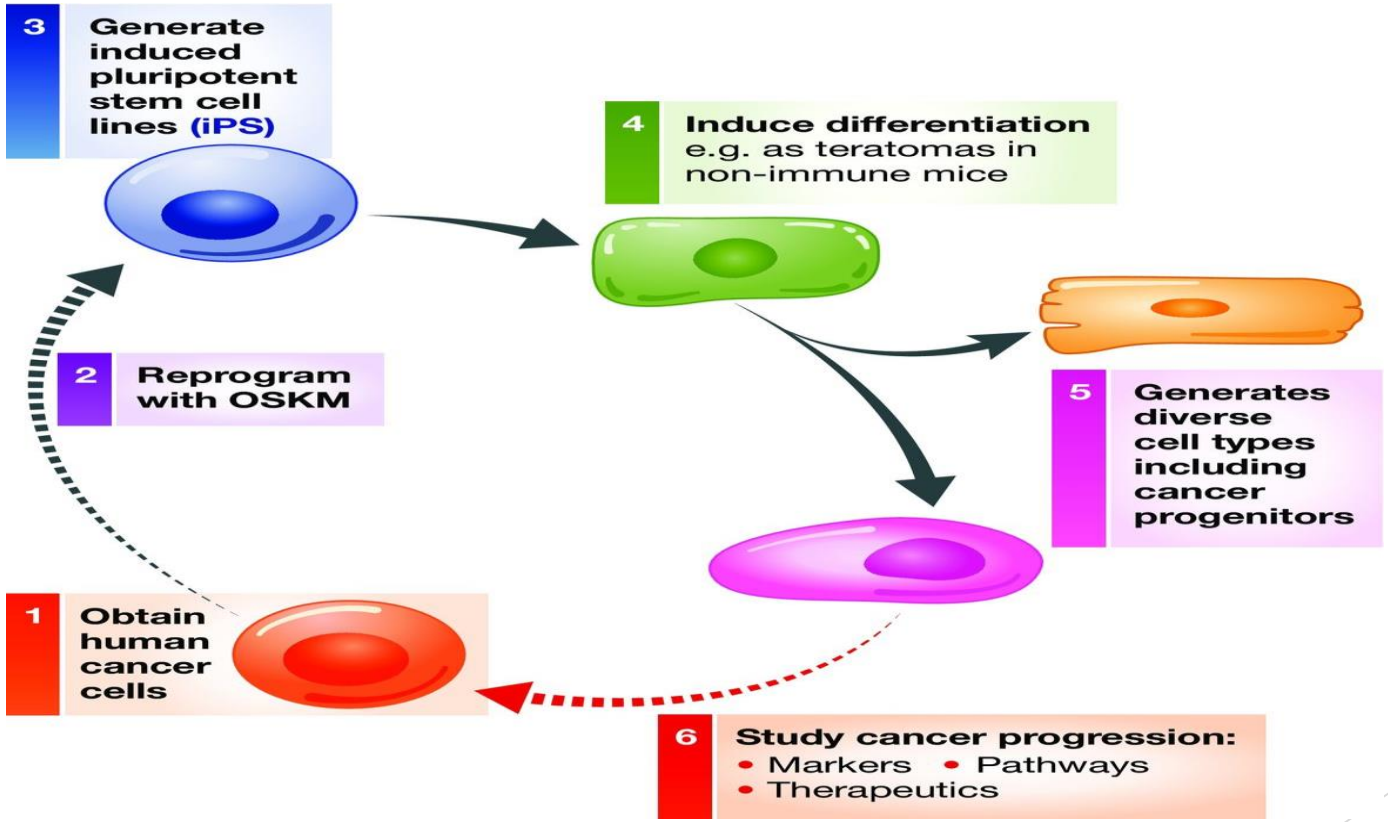
During its progression, external factors cause additional genetic and epigenetic changes

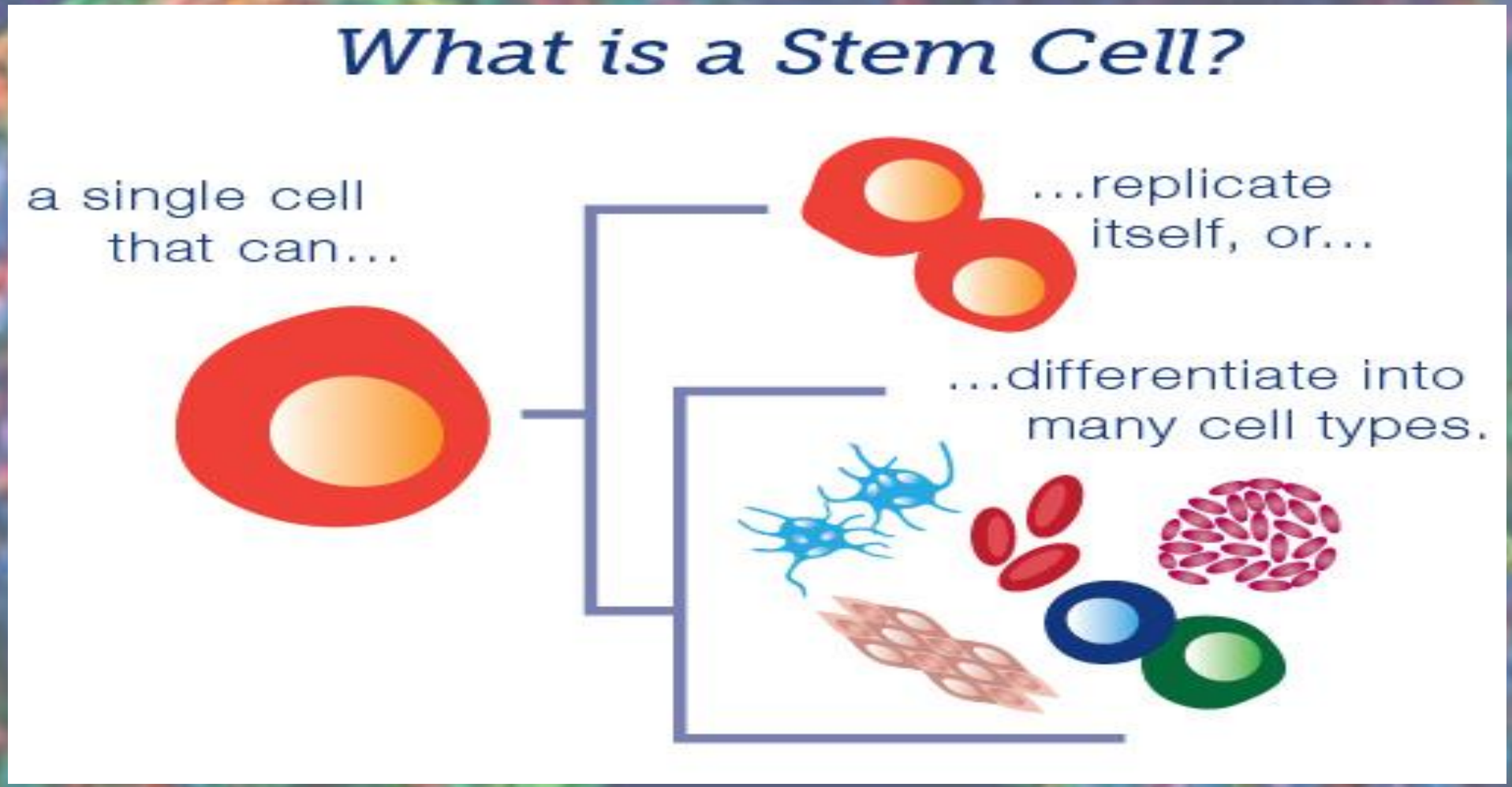
REPROGRAMING?



- first experimental of tumor reversibility(3)
- Braun, 1959
- grafted the shoots from crown-gall teratoma cells serially to the cut stem ends of the healthy tobacco plants.
- The grafted teratoma tissue gradually developed more normal appearing shoots
- ‘the cellular alteration in crown gall did not involve a somatic mutation at the nuclear gene level and rather some yet uncharacterized cytoplasmic entity is responsible for the cellular changes

cancer cells are first reprogrammed to pluripotency:





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CLASSIFICATION OF STEM CELL BASED ON POTENCY

CELL TYPE	DESCRIPTION	EXAMPLES
Totipotent	Each cell can develop in to new individual	Cells from early (1-3) days embryo
Pluripotent	Cells can form (over) cell types	Some cells of blastocyst (5 -14 days)
Multipotent	Cells differentiated but can form a number of other types	Fetal tissue cord blood and adult stem cells
Oligo potent	Ability to differentiate in to few cells	Adult lymphoid or myeloid cell
Unipotent	Ability to produce cells there own type , self renewal	Adult muscle stem cells

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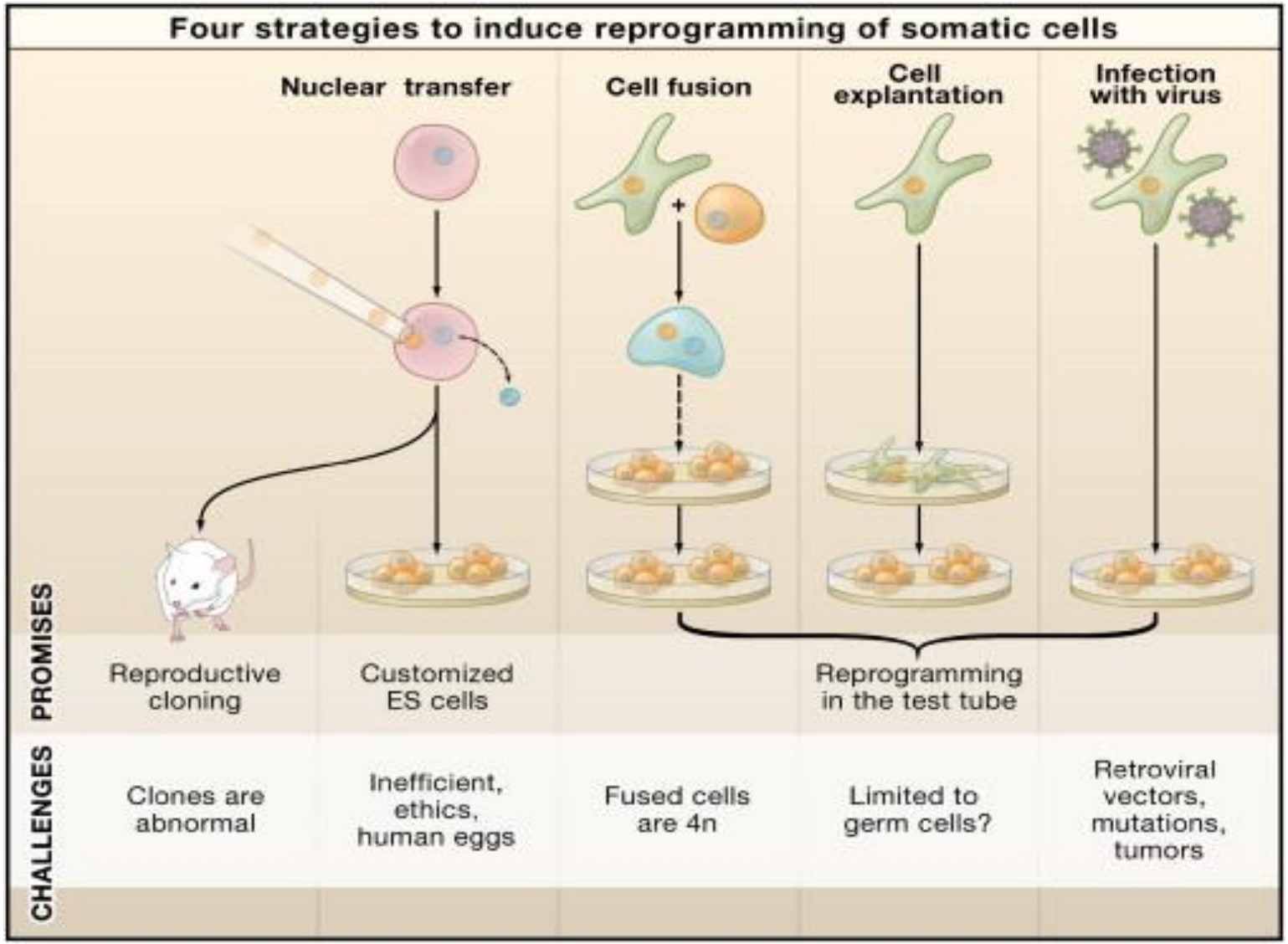


(1) Nuclear transfer(NT) injection of a somatic nucleus into an enucleated oocyte, can give rise to genetically matched embryonic stem (ES) cells ("somatic cell nuclear transfer," SCNT).

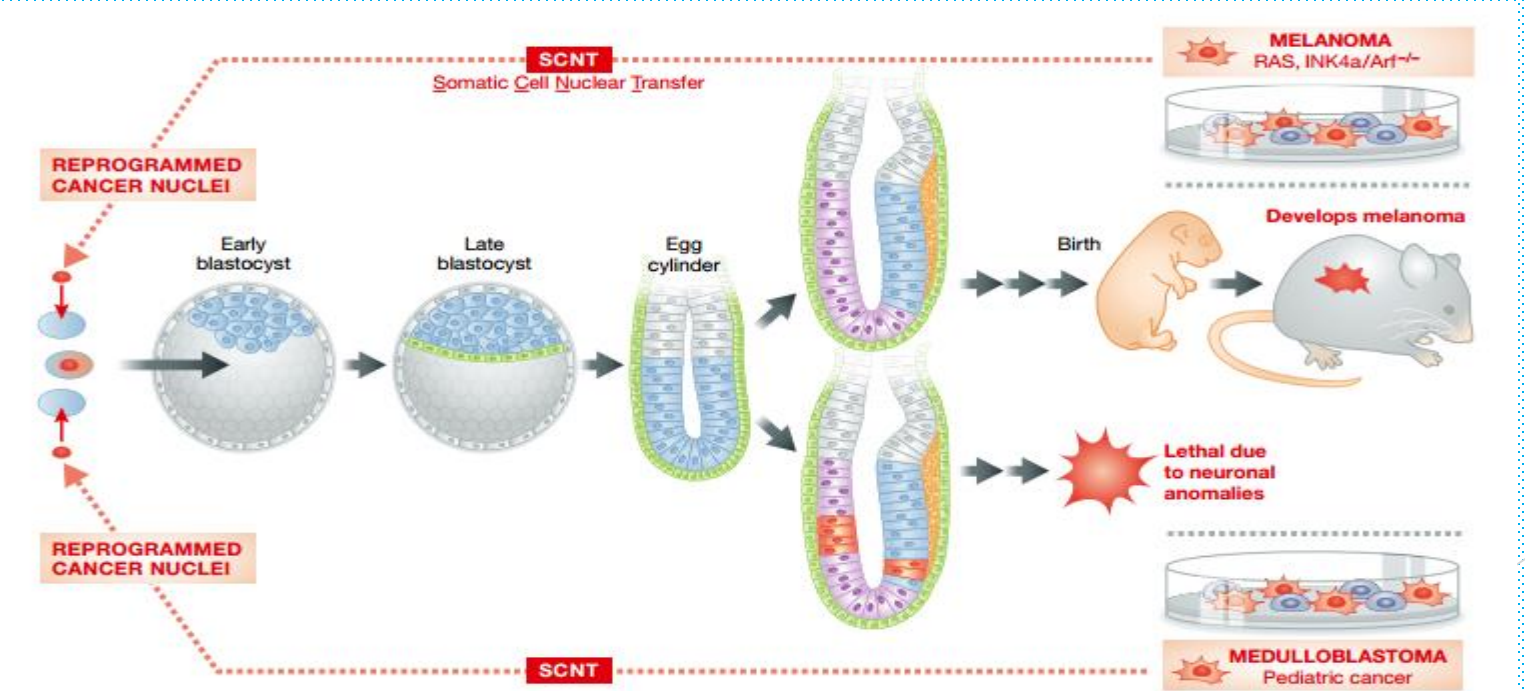
(2) a somatic cell is fused with an embryonic stem cell (ESC).

(3) Explantation of somatic cells in culture selects for immortal cell lines that may be pluripotent or multipotent.

(4) Transduction of somatic cells with defined factors can initiate reprogramming to a pluripotent state.

Nuclear transplantation (NT): (4)

- **Hochedlinger *et al(2004)***
- **whether the reprogramming activity of the oocyte can reverse the cancer phenotype of a tumor genome and establish developmental pluripotency**



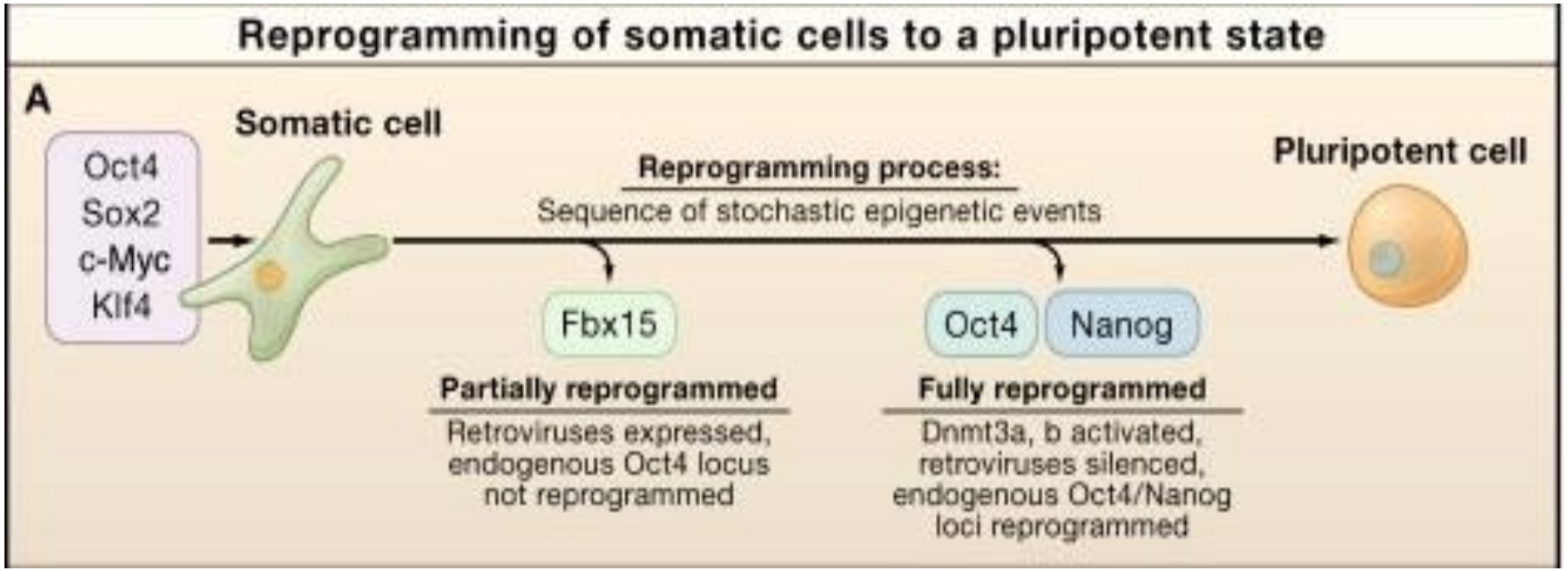
Reprogramming of a melanoma genome by nuclear transplantation:

- the nuclei of many cancer cells were able to support preimplantation development into normal-appearing blastocysts
- the malignant phenotype of these tumor types can be suppressed by the oocyte environment and permit apparently normal early development.
- Furthermore, ES cells derived from one of the cloned melanoma cells were able to differentiate into most if not all somatic cell lineages including fibroblasts, lymphocytes, and melanocytes.

Reprogramming of somatic cells to pluripotency by OSKM: (5)

- Takahashi & Yamanaka, 2006
- induced pluripotent stem (iPS) cells:
- not need oocytes or blastocyst
- somatic cells (such as fibroblasts, blood cells, etc.) reprogrammed into a pluripotent ES-like cells
- transcription factors such as Oct4, Sox2, Klf4, and c-Myc (OSKM)
- four factors must be expressed for more than 12 days in order to generate iPS cells
- epigenetic events such as chromatin modifications or changes in DNA methylation that eventually result in the pluripotent state
- much more accessible technique and it side-steps ethical issues associated with using early human embryos

fibroblasts initiates the conversion to partially reprogrammed cells that express Fbx15 or to fully reprogrammed iPS cells that express Oct4 or Nanog.



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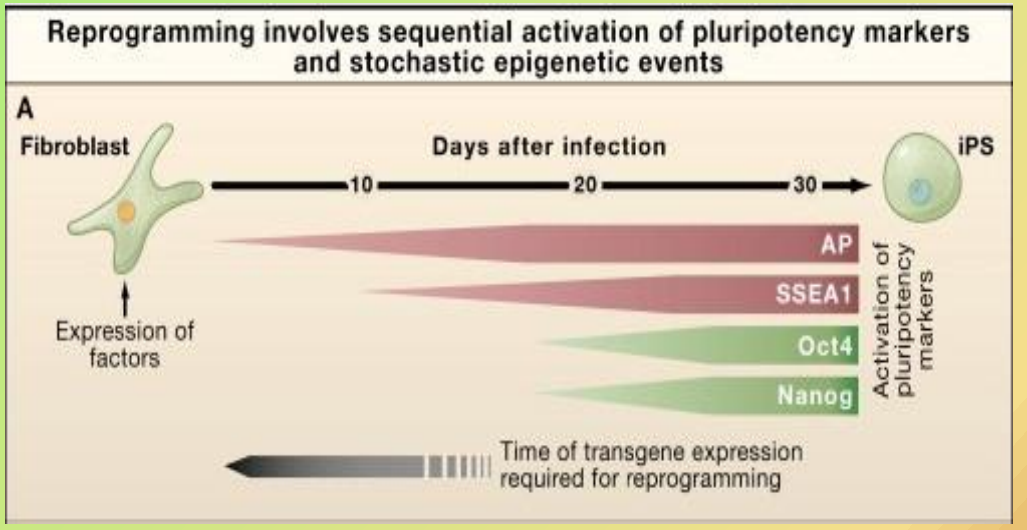
- The pluripotency of human iPS cells can be validated by cell markers, genomic RNA expression profiles, epigenetic profiles, and teratoma assays
- Reprogramming Involves Sequential Activation of Pluripotency Markers and Stochastic Epigenetic Events
- Alkaline phosphatase (AP) and SSEA1 positive cells are already detected 3 and 9 days, Oct4 or Nanog appear only after 2 weeks. The virally transduced factors need to be expressed for about 2 weeks to initiate the reprogramming process.(6)



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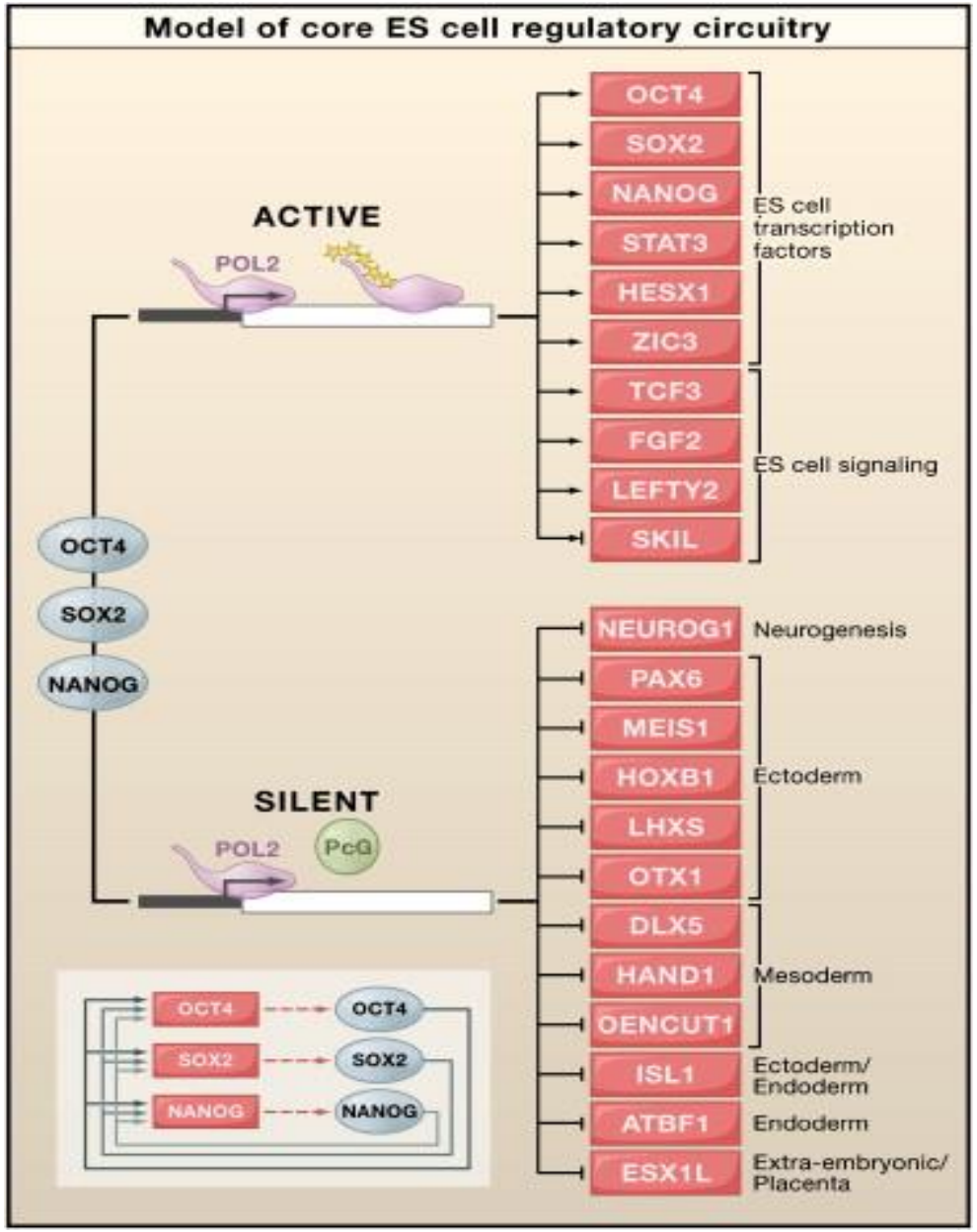
Which of the original factors are essential for the reprogramming process? (7)

- Oct4 and Sox2 are essential for pluripotency
- Nanog may function to stabilize the pluripotent state
- absence of c-*myc* transduction:low efficiency
- c-*myc* significantly enhances and accelerates the process but is dispensable
- Oct4, Sox2, and lin28, an RNA-binding protein:only obligatory factor to initiate reprogramming and that other factors serve to accelerate the process and to increase efficiency.

- transcription factors contribute to pluripotency in human and murine ES cells
- Oct4, Sox2, and Nanog are also occupied by the Polycomb group (PcG) proteins
- epigenetic regulators:
  - condensation of chromatin structure
  - gene silencing



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Reprogramming of chronic myeloid leukemia (CML): (3)

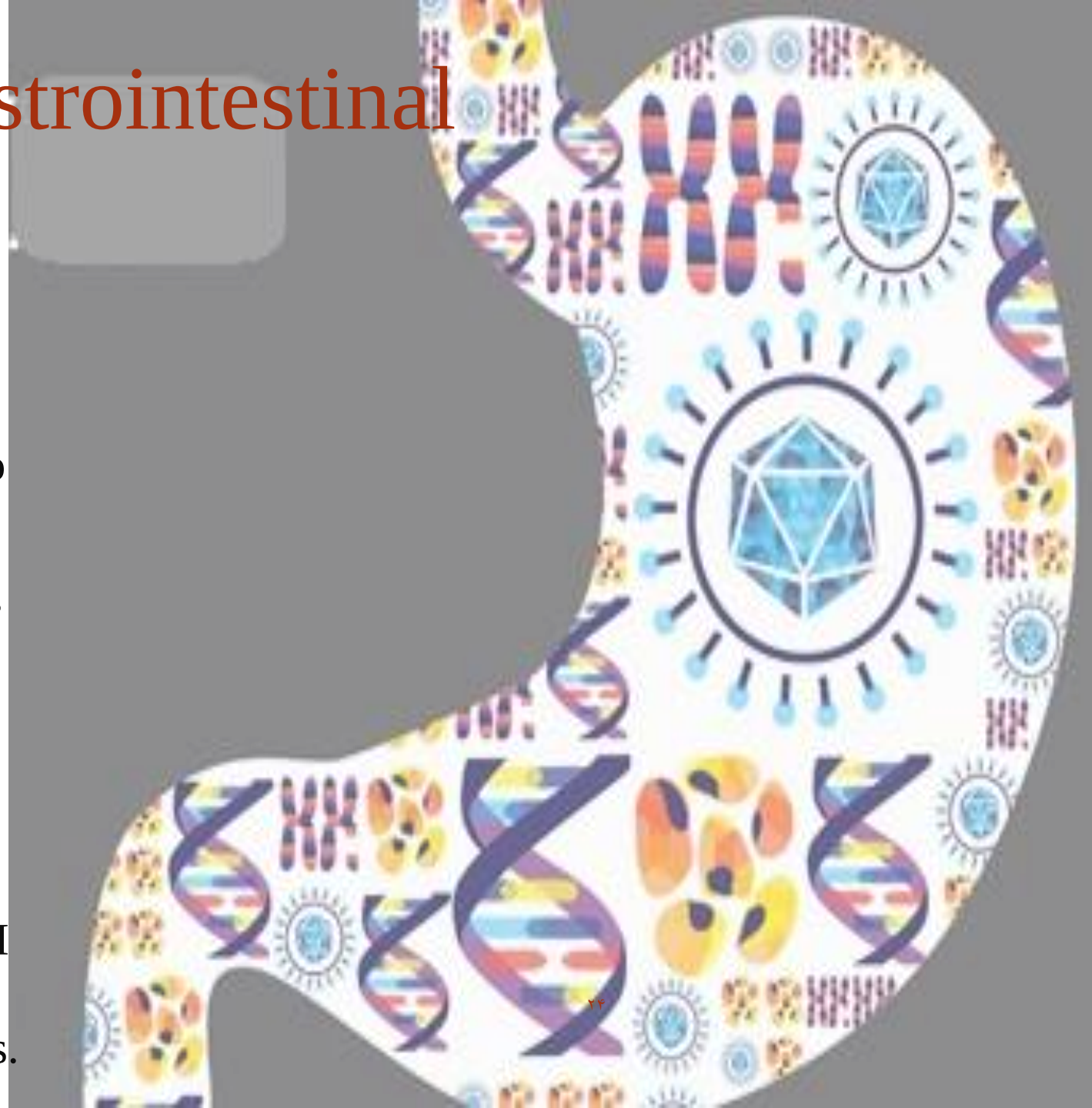
- Carette *et al* (2010)
- originates from hematopoietic stem cells of the bone marrow, is caused by a BCR-ABL fusion mutation
- dependency of CML on BCR-ABL activated tyrosine kinase
- tyrosine kinase inhibitors(TKI) : imatinib

Reprogramming of chronic myeloid leukemia (CML):

- iPSCs derived from human chronic myeloid leukemia cells
- reprogramming factors (Oct3/4, Sox2, Klf4 and cMyc)
- parental cell line was strictly dependent on continuous signaling of the BCR-ABL oncogene
- reprogrammed cells lost this dependency and became resistant to the BCR-ABL inhibitor imatinib
- oncogenic mutations can be dynamically expressed when cancer cells are converted to pluripotency and then re-differentiated.

Reprogramming of gastrointestinal cancer cell lines : (9)

- Miyoshi et al (2010)
- would allow the cells to undergo differentiation and enhanced sensitivity to therapeutics.
- gastrointestinal cancer-derived iPS cells, upon differentiation, expressed higher levels of the tumor suppressor genes p16 ^{Ink4a} and p53, slower proliferation and were sensitive to differentiationinducing treatment
- pluripotency state imposed by the OSKM factors can partially suppress the cancer phenotype in the gastrointestinal cell lines.



Reprogramming human sarcoma cell lines: (11)

- OSKM along with NANOG and LIN28
- sarcoma-iPS-derived tumors: lower grade
  exhibited more necrosis
  reduced staining for a marker of proliferation
  reduced expression of the marker than tumors from the sarcoma parental cell lines

reprogramming decreased the aggressiveness of the cancer compared to the cells' parental counterparts.

All 32 oncogenes and 82 tumor suppressor genes whose promoter DNA was initially methylated, were demethylated as a result of reprogramming, indicating that the reprogramming process was accompanied by major epigenetic changes in growth- and cancer-related genes

Reprogramming of glioblastoma (GBM) neural stem cells: (11)

- (Stricker et al, 2013).
- reprogrammed using piggyBac transposon vectors expressing OCT4 and KLF4
- GBM-iPSC into neural progenitors resulted in highly malignant cells when injected into immunocompromised mice.
- GBM-iPSC did not exhibit the malignant phenotype .
- genetic mutations render the cells malignant only when a particular cell type with the unique epigenetic state is met.

problems:

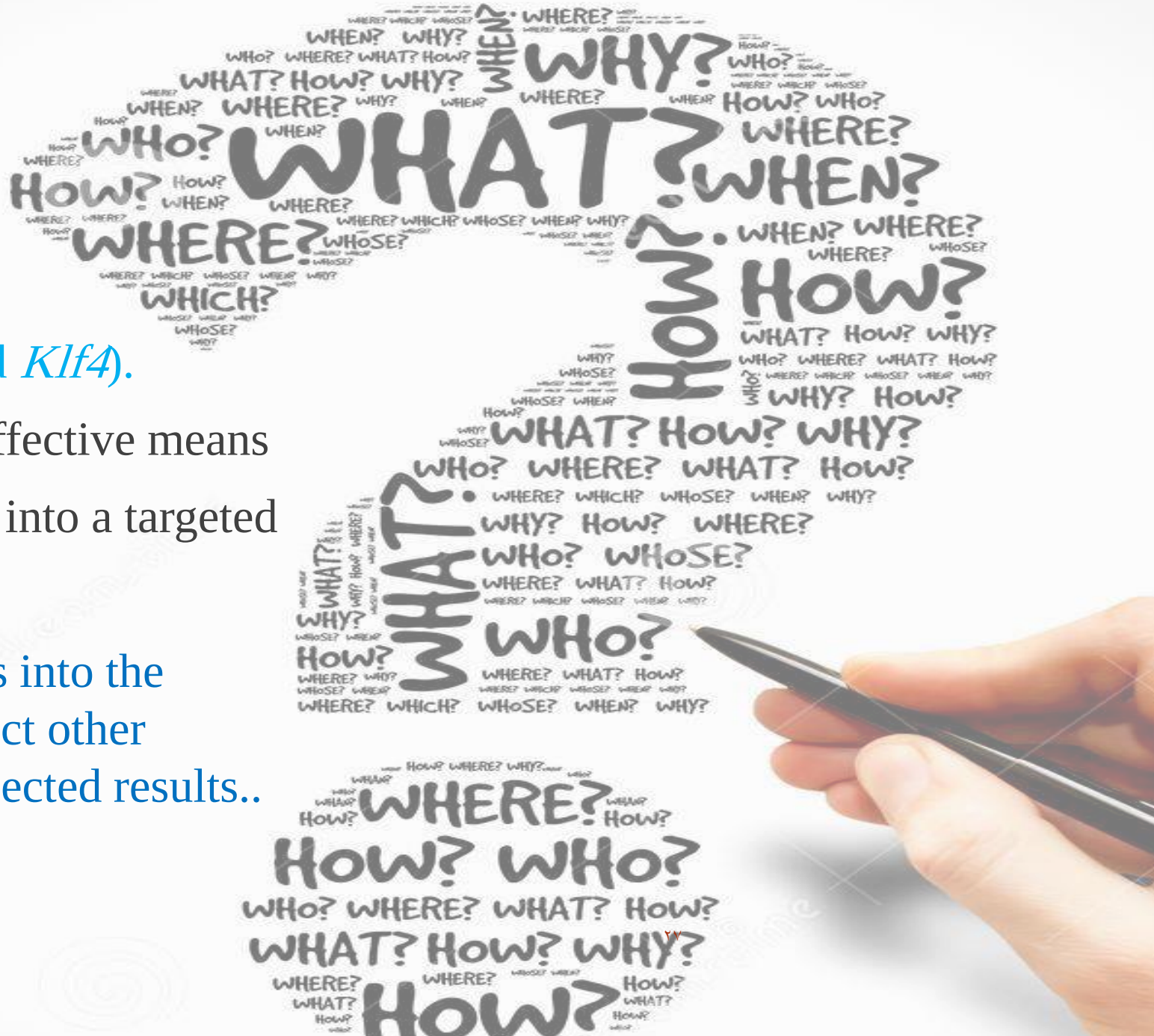
- process of iPS cell generation;

  use of retroviral transgenes

  use of oncogenes (e.g., *c-Myc* and *Klf4*).

Retroviral transfection is the only effective means to deliver the four full-length genes into a targeted somatic cell

random insertion of retroviral vectors into the transfected cell genome may also affect other nontargeted genes and produce unexpected results..



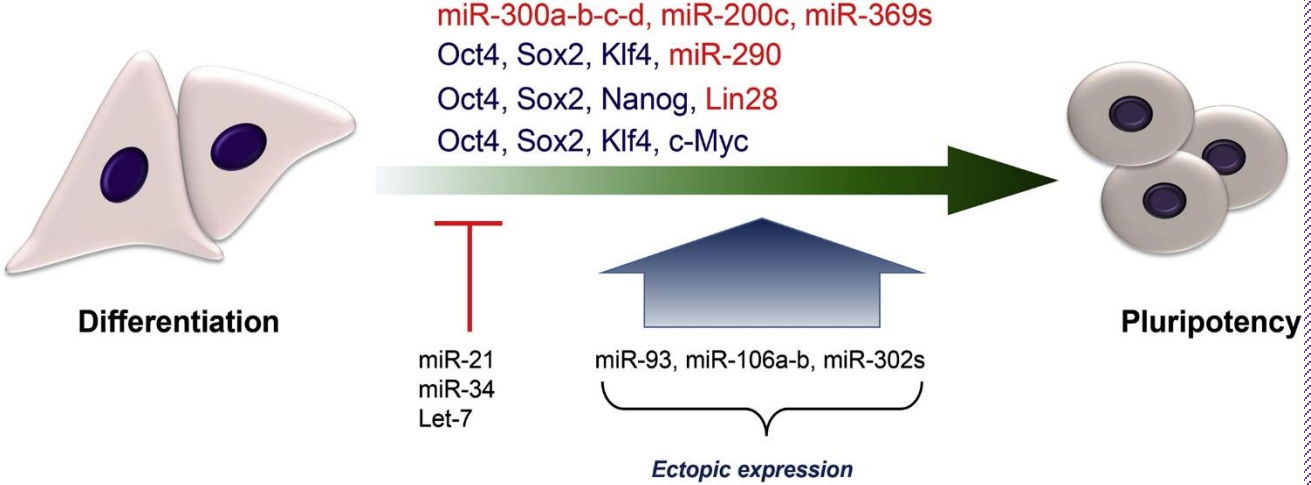
microRNA:

- small non-coding RNA molecule (ca. 22 nucleotides)
- found in plants and animals
- functions : transcriptional and post-transcriptional regulation of gene expression.
- Encoded by eukaryotic nuclear DNA
- miRNAs function via base-pairing with complementary sequences within mRNA molecules, usually resulting in gene silencing via translational repression or target degradation
- miRNAs can function as tumour suppressors and oncogenes: ‘oncomirs’

mir-302s:

mir-302s : one of the key factors essential for maintenance of ES cell renewal and pluripotency

The mir-302 microRNA (miRNA) family (mir-302s) is expressed most abundantly in slow-growing human embryonic stem (ES) cells, and quickly decreases after cell differentiation and proliferation.



MicroRNAs Induce Epigenetic Reprogramming and Suppress Malignant Phenotypes of Human Colon Cancer Cells: (12)

miR-302s and miR-369s :

induce cellular reprogramming

modulate malignant phenotypes of human colorectal cancer

Mir-302 reprograms human skin cancer cells into a pluripotent

mir-302s:

- induced epigenetic modulations of histones and DNA induction of cancer cell apoptosis(mitochondrial Bcl2 protein family.)
- attenuate the expression of their predicted target transcription factors, such as SP3, HMG-box, forkhead-box, and LIM-homeobox gene families, to provide the cell reprogramming effect..

small RNAs are unlikely to be incorporated into DNA strands in the nucleus:
reprogramming strategy may be worth considering as a novel future treatment strategy

Advantage:

- transfection of a single mir-302s–expressing transgene:very simple, efficient, and safe method
- mir-302s transgene ~1 kb:

  the transfection efficiency is extremely high (~100%)

  positive mirPS cells selected FACS flow cytometry

  transfection and cultivation of mirPS cells :feeder-free condition

  without the risk of feeder antigen contamination.
- no oncogene is used for mirPS cell generation.
- use electroporation in place of retroviral transfection to deliver the single mir-302 transgene, preventing the risk of random viral insertion into the host cell genome.

REFRANCE:

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